

WHAT IS CLAIMED IS:

1 1. A method of treating a biological material comprising the steps of:
2 (a) contacting the biological material with a preparation comprising a
3 surfactant and a cross linking agent in the absence of a denaturant; and
4 (b) contacting the biological material with a preparation comprising a
5 surfactant, a cross linking agent and a denaturant.

1 2. The method according to claim 1 wherein said method results in
2 mitigating calcification in said biological material when implanted into a host organism
3 relative to the same method that does not include step (a).

1 3. The method according to claim 2 wherein said mitigating calcification
2 results in elimination of calcification in said biological material.

1 4. The method according to claim 1 wherein said method results in
2 reducing the phospholipid content in said biological material when implanted into a host
3 organism relative to the same method that does not include step (a).

1 5. The method according to claim 1 wherein said step (a) is performed
2 prior to said step (b).

1 6. The method according to claim 1 wherein the method further
2 comprises, after completion of steps (a) and (b), contacting the biological material with a
3 terminal liquid sterilization solution.

1 7. The method according to claim 6 wherein the method further
2 comprises, prior to step (a), contacting the biological material with a solution comprising a
3 cross linking agent in the absence of a denaturant and in the absence of a surfactant.

1 8. The method according to claim 1 wherein the biological material is a
2 bioprosthetic tissue.

1 9. The method according to claim 8 wherein the bioprosthetic tissue is
2 incorporated into a bioprosthesis.

- 1 10. The method according to claim 6 wherein the terminal sterilization
2 solution comprises a cross linking agent and is heated to a temperature in the range from 35
3 to 55 degrees Celsius.
- 1 11. The method according to claim 1 wherein the cross linking agent of the
2 preparation used in step (a) and (b) is independently selected form the group consisting of an
3 aldehyde, a diisocyanate, a carbodiimide, a polyepoxy compound, a bifunctional maleimide
4 compound, a bifunctional N-hydroxysuccinimide ester compound, a bifunctional imidoester
5 compound, a bifunctional pyridylthio compound, a bifunctional vinylsulfone compound, a
6 photoactivatable cross-linkers or combination thereof.
- 1 12. The method according to claim 11 wherein the cross linking agent of
2 the preparation used in step (a) is glutaraldehyde.
- 1 13. The method according to claim 1 wherein the cross linking agent of the
2 preparation used in step (b) is a member selected form the group consisting of formaldehyde,
3 glutaraldehyde or a combination thereof.
- 1 14. The method according to claim 1 wherein the denaturant of the
2 preparation used in step (b) is a protic solvent.
- 1 15. The method according to claim 14 wherein the denaturant of the
2 preparation used in step (b) is an alcohol.
- 1 16. The method according to claim 15 wherein the denaturant of the
2 preparation used in step (b) is ethanol.
- 1 17. The method according to claim 1 wherein the surfactant of the
2 preparation used in step (a) is selected from the group consisting of a nonionic surfactant.
- 1 18. The method according to claim 17 wherein the surfactant of the
2 preparation used in step (a) is Tween 80.
- 1 19. The method according to claim 18 wherein the concentration of Tween
2 80 in is between 0.7% and 15%.

1 20. The method according to claim 19 wherein the concentration of Tween
2 80 is selected between 1.6% and 11%.

1 21. A method of treating a biological material comprising the steps of:
2 (a) first contacting the biological material with a preparation comprising a
3 surfactant, a cross linking agent and a denaturant;
4 (b) following step (a), contacting the biological material with a preparation
5 comprising a surfactant and a cross linking agent;
6 (c) following step (b), contacting the biological material with a preparation
7 comprising surfactant, a cross linking agent and a denaturant; and
8 (d) following step (c), contacting the biological material with a terminal liquid
9 sterilization solution.

1 22. The method according to claim 21 wherein said method results in
2 mitigating calcification in said biological material when implanted into a host organism
3 relative to the same method that does not include step (b).

1 23. The method according to claim 21 wherein said method results in
2 reducing the phospholipid content in said biological material when implanted into a host
3 organism relative to the same method that does not include step (b).

1 24. A biological material resistant to calcification produced by a method of
2 treating said biological material comprising the steps of:
3 (a) contacting the biological material with a preparation comprising a
4 surfactant and a cross linking agent in the absence of a denaturant; and
5 (b) contacting the biological material with a preparation comprising a
6 surfactant, a cross linking agent and a denaturant.